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Assay of "Sulfa" Ointments*

By Hertzell S. Conway

The increasing use of ointments of "sulfa" drugs, especially sulfanilamide, sulfathiazole and sulfadiazine, to prevent infection of burns and wounds requires a reliable method of assay of these preparations. The 2 parts of the assay are, first, the separation of the sulfonamide from the ointment base; and second, the determination of the sulfonamide.

Separation from Ointment Base.—Isopropyl ether has been found especially satisfactory with hydrophilic (water-washable) bases. Partition of the ointment between dilute hydrochloric acid and isopropyl ether yields 2 practically clear layers, containing sulfonamide and fatty material, respectively.

Ethyl ether can also be used as a fat solvent, but it offers several disadvantages. The extraction in separatory funnels must be carried out slowly and with proper care for the release of pressure developed by shaking. Ethyl ether is about 10 times more soluble in water than isopropyl ether, and is not so good a solvent for glyceryl monostearate and similar emulsifying agents.

Either isopropyl ether or toluene may be used to dissolve hydrophobic (nonwater-washable) ointment bases. A few analyses using heptane as the fat solvent gave low results, the reason for which has not yet been determined.

Determination of Sulfonamide.—The following procedure is recommended: To the solution of sulfonamide in 5 *N* HCl is added a small excess of standard bromate-bromide, as shown by a permanent yellow color. The excess bromine is determined by immediate addition of KI and titration with sodium thiosulfate in the usual way.

The 2 rapid, volumetric methods hitherto developed for sulfonamides are diazotization (1) and bromination (2, 3). The diazotization method is official in U. S. P. XII for pure drugs and for tablets. Its disadvantage is that it requires the use of an outside indicator (starch-iodide paste), as well as fairly close temperature control. Bromination is accurate for the pure drugs, but is inapplicable to tablets because many of the usual tablet excipients themselves consume bromine, and their removal is laborius (2, 4). On the other hand, the separation of ointment base from sulfonamide by means of isopropyl ether is rapid and easy, and the amounts of other water-soluble ingredients which may be present,

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such as glycerin, propylene glycol or the ethanolamines, do not impair the accuracy of the bromatometric assay.

When it is remembered that the usual concentration of sulfa drug in an ointment is only 5%, another advantage of bromination over diazotization becomes evident. Each sulfonamide molecule reacts with only 1 molecule of sodium nitrite, but sulfanilamide reacts with 4 atoms of bromine, and sulfathiazole and sulfadiazine react with 6. Thus samples for bromometric assay need be only one-fourth, or more frequently, one-sixth as large as samples for diazotization, in order to attain the same degree of precision with 0.1 *N* solutions.

Direct Bromine Titration Unsatisfactory.—In some other bromatometric analyses, the color of the first drop of excess bromine (5), or the destruction of an indicator dye by the excess bromine (6) can provide an accurate end point. This eliminates the need for a second standard solution for back titration. It was found here that the yellowish color of the precipitated bromination products, such as tribromosulfathiazole and tribromosulfadiazine, prevent a sharp end point by direct bromine titration, even when 0.5 *N* solution was used. Titration in the hot did not help. Addition of methyl orange as indicator dye improved the end point, but the results were still definitely higher than by back-titration. Several water-miscible solvents were tried to keep the bromination products in solution. Propylene glycol at room temperature, together with methyl orange, seemed for a time to give precise results. When, however, the results and techniques of several analysts were compared, it was found that the size of the blank (which represents mainly the reaction between bromine and propylene glycol) might vary from 0.2 to 1.0 ml. of bromine, depending inversely on the speed of titration.

Advantage of High Acidity.—In 1.5 *N* acid (such as has been recommended in former bromatometric procedures) the sulfonamide sample must stand 5 min. with excess bromine. In 5 *N* acid, the bromination is so rapid that KI may be added without any particular delay, and one avoids possible reaction of dissolved ether, hydroxylic substances or other brominatable substances which are unavoidably present. The blank in 5 *N* acid has been found to be negligible.

TABLE I. ASSAY OF "SULFA" PREPARATIONS[a]

Samples	Diazotization (U. S. P. XII)	Bromination			
		5-Min. Waiting		No Waiting	
A. Pure Sulfadiazine	100.4	100.5	100.3		100.2
	100.2	99.9	100.3		100.2
			100.1		100.3
Average, %	100.3	100.3		100.2	
B. 2.5% sulfadiazine in ethanolamines	2.58	2.51		2.51	
	2.60	2.51		2.51	
		2.51		2.47	
Average, %	2.59	2.51		2.50	
C. 5% sulfathiazole ointment (hydrophobic base)	4.81			4.84	
	4.73			4.86	
				4.80	
				5.03	
Average, %	4.77			4.88	
D. 5% sulfadiazine ointment (hydrophilic base)	4.87	4.94		4.69	
	4.82	5.06		4.70	
		5.09			
Average, %	4.85	5.03		4.70	
E. 5% sulfadiazine ointment (hydrophilic base)		5.24		5.10	
		5.21		5.17	
		5.09		5.08	
Average, %		5.18		5.12	
F. 5% sulfadiazine ointment (hydrophilic base)	5.35 5.15	5.26	5.05	4.63	4.77
	5.23 5.06	5.27	5.08	4.60	4.76
	5.14 5.07			4.61	4.84
Average, %	5.17	5.17		4.70	

[a] All ointment bases were removed by isopropyl ether extraction.

EXPERIMENTAL

Weigh quickly on a piece of cellophane or glassine paper 1.0–3.0 Gm. of ointment. Transfer to a 125-ml. separatory funnel, add 10 ml. of 1 + 1 HCl, and shake vigorously to disperse the sample. Add 50 ml. of isopropyl ether and shake again until all ointment is dissolved. After clearing, withdraw the aqueous layer into a 250-ml. Erlenmeyer flask and wash the ether layer with two 15-ml. portions of water. To the combined aqueous solutions add 20 ml. of concentrated HCl, and then a measured excess of 0.1 *N* bromate-bromide, sufficient to give a permanent yellow color. Without delay add 15 ml. of 5% KI, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate and starch indicator. Calculate the net volume of bromate-bromide consumed.

Each ml. of 0.1 *N* bromate-bromide is equivalent to: 0.004305 Gm. of sulfanilamide, 0.004255 Gm. of sulfathiazole, and 0.004171 Gm. of sulfadiazine.

RESULTS

Table I compares sulfonamide assays by diazotization; by bromination with 5-min. standing with excess bromine; and by the bromination method recommended here. Samples *B* to *F* were commercial preparations.

A and *C* indicate that for pure sulfadiazine, all 3 methods are equivalent (hydrophobic ointment base would contain no water-soluble constituents). *B* is included to show that ethanolamines do not interfere with bromination, and only slightly, if at all, with diazotization. *D*, *E* and *F* show the applicability of the recommended method to water-washable ointments. *F* seems to indicate the presence of some material which reacts quickly with nitrous acid, but slowly with bromine.

SUMMARY

A method is presented and recommended for the assay of ointments of sulfa drugs, in which isopropyl ether is used to dissolve and remove the ointment base, after which the sulfonamide is determined bromometrically. Bromination is more convenient than U. S. P. diazotization, and in the cases of pure sulfadiazine and 4 out of 5 commercial preparations gives equivalent results.

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A Brief Comparative Study of the Germination Time of Some Medicinal Plant Seeds in Flats, Coldframes and Incubators*

By Albert H. Musick†

The present world situation has increased interest in the growing of medicinal plants in the United States as a means of lessening dependence on importations, now almost negligible, and as a means of profit. Conditions of climate and soil in many foreign countries resemble those of many localities in the United States. The Puget Sound area in the state of Washington possesses the conditions of soil and climate requisite for successful growing of many medicinal plants. It was believed that it would be of interest to prospective medicinal plant growers to determine the germination time of the seeds of some medicinal plants offering commercial possibilities. For purposes of comparison the germination time was recorded on seeds grown in (*a*) flats in a greenhouse under controlled conditions of temperature, (*b*) in coldframes outside the greenhouse exposed to the variations of the weather and (*c*) in a seed germinator or incubator.

* Received Nov. 3, 1942 from the Pharmacognosy Laboratories, College of Pharmacy, University of Washington, Seattle, Wash.

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EXPERIMENTAL

Flats.—The flats used were of standard size measuring 12 x 24 inches or 10 x 20 inches in width

TABLE I.—GERMINATION RESULTS IN FLATS AND COLDFRAMES

Name	Seeds Collected	Germination Time, Days		Quantity of Germination[a]		Root System	A, B or P[b]
		Coldframe	Flats	Coldframe	Flats		
Angelica officinalis, L.	6-14-41	14	13	Very good	Good	Tap	P
Atropa belladonna, L.	10-18-33	...	35	.....	Good	Tap	P
Brassica nigra, L.	12-26-40	3	2	Very good	Very good	Fibrous	A
Brassica nigra, L.	6-24-41	3	..	Fair	.....	Fibrous	A
Carum carvi, L.	6-22-39	8	8	Very good	Very good	Tap	B
Colchicum autumnale, L.[c]	4-1-41	304	..	Fair	.....	Fibrous	P
Coriandrum sativum, L.	10-8-40	8	7	Very good	Very good	Fibrous	A
Datura stramonium, L.[e]	12-7-37	8	8	Very good	Good	Fibrous	A
Datura tatula, L.	12-18-40	9	6	Good	Good	Fibrous	A
Digitalis lanata, Ehrh.[d]	1940	12	8	Fair	Fair	Fibrous	B
Digitalis purpurea, L.	7-29-38	10	6	Good	Good	Fibrous	B
Foeniculum vulgare, Mill.	10-8-40	8	6	Fair	Good	Tap	P
Lavendula spica, L.	10-4-35	...	20	.....	Poor	Fibrous	P
Lavendula (new sp.) Metz.	10-25-40	12	8	Fair	Poor	Fibrous	P
Linum usitatissimum, L.[e]	1940	3	3	Very good	Very good	Fibrous	A
Mentha piperita, L.	11-14-40	12	8	Fair	Good	Fibrous	P
Mentha piperita, Var. *officinalis*, Sole.	11-14-40	8	8	Very good	Good	Fibrous	P
Mentha piperita (green stem)	10-30-39	12	10	Fair	Poor	Fibrous	P
Pimpinella Anisum, L.[c]	9-21-32	...	..	.....	.....	Fibrous	A
Plantago Psyllium, L.	11-14-40	6	4	Very good	Very good	Fibrous	A
Rosmarinus officinalis, L.[f]	6-24-41	...	53	.....	Poor	Fibrous	P
Salvia officinalis, L.	7-10-40	N. p.[g]	9	.....	Fair	Fibrous	P

[a] Basis of comparison (Standard = 100%); Very good = 90–100%; Good = 66–90%; Fair = 33–66%; Poor = less than 33%.
[b] A—annual, B—biennial, P—perennial.
[c] Seeds soaked in cattle guano solution.
[d] Seeds received from Switzerland in 1941.
[e] Time of collection of seed not known with certainty.
[f] Seeds insufficiently ripened.
[g] N. p.—None planted (supply exhausted).

TABLE II.—GERMINATION RESULTS IN INCUBATOR AT 86° F.

Name	Seeds Collected	Germination Time in Days
Angelica officinalis, L.	1942	8
Brassica nigra, L.	4-19-41	2
Carum carvi, L.	6-17-42	6
Coriandrum sativum, L.	7-15-41	7
Datura stramonium, L.	10-17-41	2
Datura tatula, L.	12-18-40	2
Digitalis lanata, Ehrh.	1941	7
Digitalis purpurea, L.	7-15-41	4
Foeniculum vulgare, Mill.	9-23-41	3
Lavendula nana	9-26-41	3
Lavendula (new sp.) Metz.	9-11-41	6
Lavendula vera, D. C.	9-26-41	4
Mentha piperita, L.	12-1-41	4
Mentha piperita, Var. *officinalis*, Sole (white mint)	11-14-40	2
Pimpinella anisum, L.	9-17-41	7
Plantago Psyllium, L.	11-14-40	2
Rosmarinus officinalis, L.	6-24-41	7
Salvia officinalis, L.	12-2-41	4

and length and 3 to 4 inches deep. The soil consisted of a mixture of decaying leaf mold and vegetation plus a small amount of clean fresh water sand sifted through screens. The depth of planting was governed by the size of the seeds; in general, the smaller the seed the less the depth. Seeds in a light sandy soil should be covered more deeply than those planted in a heavy clay soil.

The large seeds were either broadcast or planted in rows with soil sifted over them to a depth of 5 to 8 times their diameter. In the case of the small seed very finely divided soil was used to cover the soil. The soil was made compact and a piece of burlap placed over the flat to prevent disrupting the content upon the addition of water. The flats were then placed in an electrically controlled sand box in the greenhouse where the temperature of the ground averaged about 70° F. and the temperature of the air averaged 70 to 75° F.

Coldframes.—The soil was first broken up with a spade fork, made ready for planting and dry chicken fertilizer incorporated into the soil. The seeds were planted in rows and lightly covered with a mixture of equal parts of peat and soil. The soil was made compact and covered with burlap as for the flats.

Incubator.—The seeds were germinated in incubators at a temperature of 86° F.

SUMMARY

A comparison has been made of the germination time of the seeds of 22 medicinal plants. The comparative study was made of seeds germinating in an incubator, indoor flats and outdoor coldframes.

Many variations were observed in the germination time and quantity of growth. For the convenience of the medicinal plant grower these variations have been recorded in Tables I and II.

A Method for the Evaluation of Laxative Agents in Constipated Human Subjects, with a Study of the Comparative Laxative Potency of Fumarates, Sodium Tartrate and Magnesium Acid Citrate*

By Harry Gold and William Zahm†

The comparative potency of laxative agents has been extensively studied in lower animals. The results are, for the most part, not applicable to humans because of the differences in the reaction of various species to laxative drugs, although more recent observations show that the rhesus monkey is a suitable animal for the study of several cathartic agents (1). Human subjects with constipation should obviously yield the most direct answer to questions relating to the comparative potency of laxative agents, but, strange as it may seem, systematic comparisons in humans are almost entirely wanting.

In the present paper we describe a method for the comparison of laxative agents in constipated human subjects. The criterion for relative potency on which most reliance was placed in this study was the incidence of positive responses to a series of doses, although in the further development of the method other criteria may be added. The most precise results are secured in those cases in which all the agents are compared in one and the same person.

This method was applied in a comparative study of several organic acids. It is well known that the salts of organic acids exert a laxative action. Among the most popular are potassium and sodium tartrate (Rochelle salt, Seidlitz powders) and magnesium citrate. Gluconic acid also produces laxative effects (2, 3) in doses of 10 Gm. or more. In a previous study (4), it was found that the nephrotoxic action of fumarate in animals is negligible by comparison with the tartrate, and preliminary observations with 65 doses in 26 bed patients showed a laxative effect after 59% of the doses ranging from 5 to 30 Gm. In view of the foregoing, this study was planned to explore further the efficacy of the fumarates as laxative agents in man.

Supplementary observations on the nephrotoxic action of tartrates in cats are included.

EXPERIMENTAL

The subjects used were ambulant patients who had been in regular attendance at our clinics during periods varying from several months to several years. They were under regular social service supervision. Most of them had heart disease among other health problems. They were selected from a total case load of approximately 1500 patients. The first basis for their selection in this study was chronic constipation. There was, in all cases chosen, a long history of dependence upon a laxative such as cascara, senna, phenolphthalein, Epsom salts, milk of magnesia or mineral oil. The constipation was functional. Further classification of the constipation was not made, since that was not essential in a study of the relative potency of compounds. Its character was such that a laxative was found necessary at least twice a week, *i. e.*, it was the patient's experience that at least twice a week the daily bowel movement failed to take place at the expected time and it was their practice to secure a movement by means of a laxative agent taken at bedtime of that day or the following morning. A few subjects were included who required a laxative only once a week, and also a few in whom constipation was so obstinate that a laxative was used daily.

The laxative agent under investigation was taken following the lapse of several hours after the expected daily bowel movement (always 24 hrs. or longer since the last bowel movement). In the majority of cases it was taken in the morning. This secured a more satisfactory record of the period required for its effect. A few subjects preferred it at bedtime.

A simple record form was issued at every clinic visit made at intervals of 1 or 2 weeks. It contained the following rubrics: day of the week, time medicine was taken, time of the bowel movement, kind of

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† The authors are indebted to Miss Ida Dailes for technical assistance in the conduct of this study.

bowel movement (normal, loose, hard, watery). The patient filled this out at the time and returned it during the subsequent visit to the clinic.

Five compounds were studied: sodium fumarate, magnesium fumarate, calcium fumarate, sodium tartrate and magnesium acid citrate. They were dispensed in powders containing 10 Gm. each, and in quantities sufficient to last 1 or 2 weeks in different cases. The powder was taken dissolved in one-half glass of water followed by one-half glass of water. Calcium fumarate and magnesium fumarate are not very soluble and made a slightly bitter partial suspension. The remaining 3 compounds made a clear salty solution.

The plan of the study involved the assumption that if a patient took a fixed dose of a laxative agent for constipation, and repeated the dose as the conditionally the positive result occurred within a few minutes, in a few cases nearly 24 hrs. elapsed. There is a strong probability that in the first case the effect resulted from a reflex rather than from the direct action of the agent, and in the second case the bowel movement may have been unrelated to the drug. The possibility of spontaneous bowel movement after the drug, independent of any drug action, is always there. We have not determined how great a source of error this might be, but since the number of cases is large and such an error should apply equally to all the preparations, it is probably of no importance in the deductions regarding the relative potency of the various compounds.

The 10-Gm. dose was selected for this study, since preliminary observations with fumarate (4) showed that it was not uniformly effective and yet gave a

TABLE I.—ILLUSTRATIVE TYPE OF DATA

Order of Doses / Drug	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Patient N. M.																
Sodium fumarate	0[a]	7.5	0	0	0	0	0	2	1.5	1.5	...	...	...	...	...	...
Calcium fumarate	3	3	3	3	3	3	0	2.5	2.5	...	...	...	...	...	...	...
Magnesium fumarate	1.5	1.5	1.5	2	2	2	1.5	1.5	1.5	1.5	2	2	...	...	...	...
Sodium tartrate	1.5	1.5	1.5	1	1	1	1	1	1	...	...	...	...	...	...	...
Magnesium acid citrate	2.5	0	2.5	0	1.5	1.5	2	2	2	0	1.5	2	...	...	...	...
Patient A. J.																
Sodium fumarate	1	7	18.5	1.5	4	0	2	2	11	...	...	...	...	...	...	...
Calcium fumarate	8	4	2	17	3	5	16	1	12	...	...	...	...	...	...	...
Magnesium fumarate	0	4	3	1	3	7	2	5	3	1	3	4	10	...	...	...
Sodium tartrate	0	3	10.5	3	2.25	20	6	8.5	2.5	3	...	...	...	...	...	...
Magnesium acid citrate	6	1	1	3	4	13	1	4	5	12	5	4	...	...	...	...
Patient M. S.																
Sodium fumarate	12	1.5	1.75	3.5	0	2.5	0	1.25	3	0	...	...	...	...	...	...
Calcium fumarate	0.5	1.25	0	6	0.3	2.5	0	4	0.75	0	0	7.5	2	0	0	1.3
Magnesium fumarate	0.6	0	1.25	1.5	0	0	0	1.5	0	0.5	0	0.5	0.5	2	...	...
Sodium tartrate	0	1.5	1.5	0	2	0	2	2	3.5	0	0	2.5	5	0	...	...
Magnesium acid citrate	0	2	0	0	0	0	0	0	0	1.5	0	0	0	0.5	1	2

[a] The zeros indicate failure of the dose; other figures indicate number of hours to a positive response.

tion recurred at intervals over a period of 3 to 4 weeks, the incidence of positive responses to the dose would vary with the potency of the laxative agent. While we have not determined the sensitiveness of the method or the character of the dosage-response curve for the agents in question, there is sufficient general experience to insure the fact that the basic assumption is valid. We have also assumed that if the standard preparation with which comparisons were to be made yielded positive responses considerably below 100%, practical differences in the potency which might exist between the compounds under investigation would be disclosed.

The response was considered positive if the bowel movement took place within 24 hrs. after the dose. There is here without doubt a source of error. Occa-

fairly high incidence of positive responses. In the case of potassium and sodium tartrate, 10 Gm. has been established as the average laxative dose on the basis of general experience. A smaller dose might have been nearer to the more sensitive part of the dosage-response curve, but might also create a more difficult problem, since it would not be feasible to carry out protracted experiments in patients with a dose of a laxative agent which was too often ineffectual.

RESULTS

The study was started with 256 subjects. Since fairly rigid criteria were maintained for their continued service, a great many were dropped for various reasons and the final analyses were made from the results obtained with 140 subjects, or 55% of the

original group. The remaining 45% were discontinued for such reasons as these: inability to keep an accurate record, inability to write English, preference for their customary laxative, prejudice against the use of a powder, periods of constipation too irregular, etc. Among the final group both sexes were represented and the ages ranged from 23 to 76 years, average 56.6 years. It should be noted that these patients constitute a very satisfactory group of subjects for long-range studies on ambulant patients. A large proportion of them were familiar with the technique of coöperation in long-period systematic treatments, having served as ambulatory patients in the studies of the xanthines and digitalis, in which they had grown accustomed to reporting effects systematically and accurately.

Table I shows the type of data in 3 representative cases. Each of these patients received a series of doses of each of the 5 compounds. In some cases, one agent produced more positive responses, while in other cases the incidence of positive results was essentially similar for all agents.

TABLE II.—SUMMARY OF EFFECT OF 10-GM. DOSES OF 5 LAXATIVE AGENTS IN A GROUP OF 140 PATIENTS

Drug	No. Patients	Total No. Doses	Average No. Doses per Patient	Results No. Pos.	Results No. Neg.	Results % Pos.	Time (Hrs.) for Pos. Result Range	Time (Hrs.) for Pos. Result Average
Sodium fumarate	93	688	7.39	493	195	71.6	0.08–23.50	6.90
Calcium fumarate	61	451	7.39	315	136	69.8	0.33–23.75	6.46
Magnesium fumarate	61	540	8.85	442	98	81.8	0.33–22.00	6.09
Sodium tartrate	43	379	8.81	308	71	81.3	0.50–23.75	5.45
Magnesium acid citrate	38	335	8.81	264	71	78.8	0.50–23.50	7.03

person to person. However, the experiments were not designed to establish this point.

In order to determine whether these differences are significant, the data have been subjected to more detailed statistical analysis for which we are indebted to Dr. C. I. Bliss. The statements which follow are based on his findings.

For the incidence of positive responses, the analysis was based only on cases with 4 or more doses of any one agent. The results are presented in Table III. The patients were divided into 2 groups: Group I represents 13 patients who received all 5 drugs and in whom the reactions were neither all positive nor all negative to all drugs; Group II represents the additional patients tested with 1 to 4 of the drugs, including also those with all positive or all negative responses to the 5 drugs (4 such patients).

There were 17 patients who were tested with all 5 agents. Four of these patients were omitted from

TABLE III.—ANALYSIS OF DATA RELATING TO THE INCIDENCE OF POSITIVE RESPONSES WITH THE 5 LAXATIVE AGENTS

Group		Sodium Fumarate	Calcium Fumarate	Magnesium Fumarate	Sodium Tartrate	Magnesium Acid Citrate
I	Average no. of doses per patient	12.3	11.8	12.8	11.8	12.0
	Percentage of 13 patients with no failures	23.1	30.8	30.8	38.5	46.2
	Mean[a] percentage of positive responses for all 13 patients	72.7	75.6	79.3	78.3	81.7
II	Total number of patients	57	37	43	26	22
	Percentage of patients with no failures	38.6	37.9	55.8	61.5	54.5
	Percentage of patients with all failures	14.0	13.5	11.6	7.7	18.1
	Number of patients with some failures	27	18	14	8	6
	Mean[a] percentage of positive responses, based on patients with some failures	65.4	53.5	68.6	65.6	57.1

[a] Mean of percentages for each patient, each weighted by square root of number of doses of each drug.

While it would have been most desirable to make all comparisons in one and the same person, such comparisons proved feasible in only a small number of the total group. In Table II are summarized the results with the entire group of 2393 doses in 140 patients. The number of doses taken by the different patients varied greatly. Some of them took only 1 dose, others took as many as 100 doses from

Group I, one because none of the doses produced a positive reaction, and the other 3 because all doses were effective. The variance between patients was over 5 times the error variance and highly significant, indicating the importance of balancing differences between individuals in such experiments. Because of this, the averages in Table III were computed from the percentages for each patient rather than from the total number of doses and the total number of positive responses, weighting each percentage so as to compensate approximately for the differences between the number of doses per patient, which varied for a single drug from 4 to 26. Differences in the incidence of positive responses between the 5 drugs in these 13 patients were not significant when tested in an analysis of variance.

In Group II, which consists of the additional series of patients tested with from 1 to 4 of the drugs, the "sensitive" patients who always responded and the "insensitive" ones who always failed to respond have been listed separately and excluded from the calculation. The mean percentage of positive responses for the different drugs was computed only from those patients in whom the incidence of positive responses fell between the foregoing extremes. Because individual differences could be only partially balanced at best in this series, the results are more irregular, despite the larger number of patients.

As already stated, the chief criterion for comparing the potency of the 5 laxative agents was the incidence of positive responses to a fixed dose. It is possible, however, that other criteria might be used, such as the percentage of patients who will show all failures or no failures in response to a fixed dose of the various agents. In Table III a comparison of the column of figures for the percentage of patients with no failures in Group I with that showing the mean percentage of positive responses, reveals 2 parallel trends, a tendency for a rising incidence of patients with no failures, running parallel with a slightly rising incidence of positive results as one reads across from sodium fumarate to magnesium acid citrate. This suggests that there may be some difference in the potency of the group of agents studied, but the difference is so small that in an analysis of variance of our data, as already indicated, it is not significant. The reverse order of some of these differences in Group II of Table III further suggests that no substantial difference in the potency of these compounds exists.

The data concerning the length of time required for the laxative effects for the 5 compounds have been similarly analyzed. The length of time for an effect might also be used as a criterion for comparing the potency of laxative agents. From some standpoints this criterion possesses advantages as a quantitative index of potency over the criterion of percentage of positive responses. The results are presented in Table IV. Here again the patients were divided into 2 groups: Group I includes those patients in whom all 5 agents were used; Group II comprises additional patients in whom 1 to 4 of the agents were used. The analysis of variance shows that there is no difference between the 5 drugs used with respect to the length of time that elapsed between the dose and the laxative effect. This analysis was made in the case of the 16 patients (Group I) who were tested with all 5 drugs. The variability between patients is again very large, larger than in the case of the other criterion (percentage of positive responses), its variance being here more than 42 times as large. With this criterion, it is therefore especially important to balance differences between patients. Exclusive of differences between patients, the correlation between the percentage of positive responses and the mean log-time was small and not significant ($r = 0.211$, $M = 47$). Despite this

TABLE IV.—ANALYSIS OF DATA RELATING TO THE TIME REQUIRED FOR THE POSITIVE RESPONSES WITH THE 5 LAXATIVE AGENTS

Group		Sodium Fumarate	Calcium Fumarate	Magnesium Fumarate	Sodium Tartrate	Magnesium Acid Citrate
I	No. of patients with all doses positive	6	7	7	8	9
	No. of patients with some failures	10	9	9	8	7
	Mean[a] time in hours in patients with all doses positive	3.30	4.11	4.90	3.61	4.35
	Mean[a] time in hours in patients with some failures	7.06	5.93	4.48	5.74	6.05
	Mean[a] time in hours in all patients	5.05	4.90	4.68	4.52	4.98
II	No. of patients with all doses positive	22	13	25	14	10
	No. of patients with some failures	20	13	11	7	4
	Mean[a] time in hours in patients with all doses positive	2.71	4.52	3.83	3.85	5.04
	Mean[a] time in hours in patients with some failures	6.54	5.73	5.95	4.58	9.71

[a] Geometric means based on average for each patient and drug, weighted by square root of number of positive responses in each individual average.

TABLE V.—TYPES OF STOOLS WITH THE 5 LAXATIVE AGENTS

Drug	Total No. Positive Doses	Normal No.	Normal %	Hard No.	Hard %	Diarrheal[a] No.	Diarrheal[a] %
Sodium fumarate	493	175	35.5	184	37.3	134	27.2
Magnesium fumarate	442	187	42.3	69	15.5	186	42.2
Calcium fumarate	315	133	42.2	109	34.6	73	23.2
Sodium tartrate	308	143	46.4	52	16.9	113	36.7
Magnesium acid citrate	264	138	52.3	71	26.9	55	20.8

[a] Loose, watery or frequently repeated.

small correlation, the geometric mean interval for the laxative effect was consistently and significantly ($P < 0.01$) shorter in patients who showed a positive response to all doses than in those who showed some failures with the given drug (1 exception).

The data relating to the type of stools have been assembled in Table V. It may be noted that normal, hard, and watery or diarrheal stools are represented among the doses of all five preparations. The patient's judgments concerning the type of stool are not sufficiently precise to warrant a statistical analysis of the differences indicated in the table.

The data concerning disagreeable effects such as nausea and/or vomiting, and abdominal cramps have been assembled in Table VI. It is clear that similar disagreeable effects are produced by all 5 compounds and that the gross data indicate some significant differences in the incidence among the 5 drugs. It was not found feasible to analyze these data with respect to differences between patients, and although differences between the drugs are suggested in an analysis based on the total number of doses, it is considered unsafe to draw any inference from this analysis in view of the well-known wide differences between individuals with regard to such reactions.

TABLE VI.—DISAGREEABLE EFFECTS WITH THE 5 LAXATIVE AGENTS

Drug	Total No. Doses Administered	Nausea and/or Vomiting No.	Nausea and/or Vomiting %	Cramps No.	Cramps %	Total of Disagreeable Effects, %
Sodium fumarate	688	56	8.2	20	2.9	11.1
Magnesium fumarate	540	21	3.8	18	3.3	7.1
Calcium fumarate	451	31	6.8	9	1.9	8.7
Sodium tartrate	379	6	1.6	8	2.1	3.7
Magnesium acid citrate	335	2	0.6	11	3.2	3.8

Nephrotoxic Action of Tartrates in the Cat.—In a previous study (4) it was shown that 300 mg. of sodium tartrate per Kg. given intravenously in a cat produced severe renal damage, as shown by the fall of the phenolsulfonphthalein excretion to zero, and marked elevation of the blood N. P. N. and creatinine. The histological sections of the kidney

TABLE VII.—EFFECT OF SODIUM TARTRATE ON THE BLOOD AND URINE IN CATS

Cat. No.	Dose Single, Mg./Kg.	Dose Total No.	Total Dose Gm./Kg.	Experimental Day[a]	Blood N. P. N., Mg. %	Blood Creatinine, Mg. %	Urine Alb. and Sugar	Urine Spec. Grav.
7	Control		...	2	33	1.4	0	...
(Original wt. = 3.0 Kg.			...	8	38	1.5	0	1.054
Final wt. = 3.1 Kg.)			...	13	36	1.5	0	1.076
			...	31	38	1.3	0	1.050
			...	47 to 48	38	1.7	0	1.046
			...	68	41	1.4	0	1.040
			...	82	42	1.6	0	1.050
			...	96	39	1.5	0	1.044
			...	110	41	1.4	0	1.050
			...	126	44	1.2	..	...
4	100	45	...	−16	43	1.7	0	...
(Original wt. = 3.0 Kg.			...	−13	40	1.6	0	...
Final wt. = 3.1 Kg.)			...	−8 to −1	39	1.6	0	1.070
			0.5	+12	38	1.1	0	1.040
			1.1	+26	53	1.7	0	1.040
			1.5	+34	54	0.9	..	...
			2.0	+46	47	1.4	0	1.058
			2.6	+61	44	1.6	0	1.045
			3.2	+75 to +77	43	1.3	0	1.036
			3.8	+89	44	1.3	0	1.050
			4.5	+105	46	1.2	..	...

[a] (−) indicates number of days before injections were started; (+) indicates number of days after beginning of the injections.

in this animal showed marked tubular degeneration. Several times that dose of sodium fumarate was without this effect. The question arose whether small doses of tartrate might not also, by frequent repetition, give rise to renal damage, even though the doses were insufficient to cause chemical changes in the blood. This question was tested in a group of 8 cats, 3 used as controls, and 5 receiving sodium tartrate. In 1 cat the dose was 50 mg./Kg., in each of 2 others 75 mg./Kg., and in each of the last 2, 100 mg./Kg. The drug was given in a 5% solution of sodium tartrate by subcutaneous injection 3 times a week for a total of 45 doses over a period of nearly 4 months. A summary of the data for a typical experiment suffices to illustrate the plan of the study and the results (Table VII).

The animals were maintained on a constant diet of the following: 150 Gm. of canned dog food 3 times a week, 150 Gm. of fresh meat twice a week and 150 Gm. of fresh liver once a week. The controls, as well as the treated animals, maintained their body weight throughout the period of study. There were no significant changes in the N. P. N. or creatinine. The animals were sacrificed at the end of the experiment. The weights of the kidneys were essentially the same for the treated and the control groups, 12 Gm. each (11 to 13.5 Gm.) for the treated, and 11 Gm. each (10 to 16 Gm.) for the controls. The kidneys appeared similar on gross examination. The kidneys of 4 animals were examined histologically. The sections of the 2 control animals were normal, while those of the 2 animals which received the 100-mg. doses of sodium tartrate showed considerable tubular degeneration. These doses for cats are substantially similar to the doses of tartrate that would pass through the kidneys of a person after a 20-Gm. dose taken orally. These results suggest a potential hazard in the continued use of tartrates as laxatives, which could easily escape detection.

SUMMARY AND CONCLUSIONS

1. A method is described for the quantitative comparison of laxative agents in constipated human subjects.

2. By this method it is shown that the laxative efficacies of the fumarates of sodium, calcium and magnesium are the same, and that these three also possess approximately the same laxative potency as sodium tartrate and magnesium acid citrate, gram for gram. A 10-Gm. dose of any of the foregoing produces a bowel movement within an average of about 6 hrs. in about 75% of the times it is used in patients in need of a laxative.

3. The fumarates provide satisfactory substitutes for the tartrates as laxative agents, without the potential nephrotoxic hazards of the tartrates.

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Pharmacologic Action of Gelsemine and Dihydrogelsemine*

By F. G. Henderson and K. K. Chen

The alkaloid gelsemine has been known for over seventy years. It has been isolated repeatedly from the roots and rhizomes of the yellow jasmine, *Gelsemium sempervirens*. Earlier reports were made by Wormley (1), Spiegel (2), Goeldner (3) and Moore (4). Chou (5), working in Paris, succeeded in obtaining three crystalline substances from an American sample of *Gelsemium sempervirens* one of which was gelsemine. The empirical formula of the latter was found to be the same as that of Moore, namely, $C_{20}H_{22}O_2N_2$. Recently Chu and Chou (6) prepared dihydrogelsemine by catalytic reduction. A preliminary report on the pharmacology of gelsemine, based upon the work of one of us (K. K. C.), was made by Chou (5), but dihydrogelsemine has not been investigated in animals.

An unsaturated linkage sometimes has a profound influence on the pharmacologic action. For example, allyl alcohol is much more toxic than propyl alcohol. Among the digitalis-like substances a saturation of the double bond results in loss of activity

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(7, 8). It has been shown that 2-methyl-ethyl barbituric acid is shorter acting than 2-methyl-propyl-ethyl barbituric acid (9). On the other hand, dihydro-diethylstilbestrol and diethylstilbestrol have about the same degree of activity when given by mouth, although the former appears to be more active by injection (10). It is thus of interest to compare gelsemine and dihydrogelsemine from the pharmacologic point of view. Both of the alkaloids, in the form of the hydrochlorides, were courteously supplied by Dr. T. Q. Chou, of the Institut de Materia Medica, Université Franco-Chinoise, Shanghai, to whom we are much indebted.

To strips of isolated rabbit's intestines suspended in 100 cc. of Locke-Ringer's solution, an amount of 8.2 mg. each of gelsemine hydrochloride was added. Relaxation of peristaltic movement promptly followed. In similar experiments dihydrogelsemine hydrochloride in the concentration of 1:10,000 also produced intestinal inhibition.

The action upon the isolated rabbit's uterus, suspended in Locke-Ringer's solution, was studied with both alkaloids. A concentration of 1:20,000 of each caused contraction.

In etherized cats, dihydrogelsemine hydrochloride in the dose of 5 mg. injected intravenously, lowered slightly the carotid blood pressure. An amount of 9 mg. produced a slight rise followed by a fall, and one of 18.5 mg. had a depressor action only. On account of the limited supply of gelsemine no experiments were performed with that alkaloid for the observation of blood pressure. However, it has already been demonstrated that gelsemine hydrochloride causes a fall of blood pressure (5).

The toxicity of gelsemine hydrochloride and dihydrogelsemine hydrochloride was determined in albino mice by intravenous injection of 0.5% solutions of each. The median lethal doses (LD_{50}) of the two alkaloids are approximately the same as shown in Table I. The slight difference is statistically insignificant.

TABLE I.—COMPARISON OF TOXICITY OF GELSEMINE HYDROCHLORIDE AND DIHYDROGELSEMINE HYDROCHLORIDE IN MICE BY INTRAVENOUS INJECTION

Drug	Dose, Mg./Kg.	Number Dead/Number Used	$LD_{50} \pm$ S. E., Mg./Kg.
Gelsemine hydrochloride	80	0/1	103.5 ± 4.36
	100	3/7	
	110	3/5	
	125	5/5	
Dihydrogelsemine hydrochloride	90	0/5	104.8 ± 1.84
	100	2/16	
	110	8/10	

From the above data, it seems that the pharmacologic action of gelsemine closely resembles that of its reduced form, dihydrogelsemine. Both alkaloids relax the rabbit's intestinal muscle and both contract the isolated rabbit's uterus, lower arterial blood pressure in cats, and show the same toxicity in mice.

CONCLUSION

Quantitatively and qualitatively, gelsemine hydrochloride and dihydrogelsemine hydrochloride have a similar pharmacologic action.

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Effects of Age and Sex upon the Margin of Safety of "Delvinal" Sodium Vinbarbital and of Calcium 5-Ethyl 5-(2-Butyl) N-Methyl Barbituric Acid in the Albino Rat*

By Harald G. O. Holck, James R. Weeks, Donald R. Mathieson and Beatrice Duis

The *margin of safety* or *therapeutic index* has usually been expressed for barbiturates by the ratio between a more or less well-defined fatal dose and a dose which produced a certain degree of depression in the animal as a whole or abolition of a specified reflex. Girndt (1) has prepared an elaborate plan by which different degrees of depression may be evaluated. In order to convey more complete information about a drug in question, some authors (2, 3, 4, 5, 6) have given several criteria for fatality or depression in their publications. In recent years it has become customary to compare the dose lethal to 50% of the animals (LD_{50}) with the dose effecting the given degree of depression in 50% of the animals (ED_{50}). However, Foster (7) has urged that the LD_1, the dose killing but 1%, which is close to the maximum tolerated dose, be compared with the ED_{99}, the dose causing hypnosis, anesthesia or other given degree of depression in 99%, or practically all the animals, and has presented a method for use in such calculations.

In some cases no mention is given in the literature as to the age or size of the rats, in others it is stated that the rats were adults or young adults, or an average weight is given. Presumably, in comparative studies, rats of approximately the same age were employed. Although distinct sex differences in mortality and duration of hypnosis repeatedly have been reported for the rat toward various short-acting barbiturates, many authors fail to mention the proportion of the sexes, though some state this proportion, or that only one specified sex was used in their tests.

Inasmuch as we have reported recently that young rats, as well as adult males, develop tolerance more readily than adult females (8), and because even with the same barbiturate the safety margin varied in different reports, we decided to investigate the effects of age and sex upon this ratio. This desire was strengthened by the fact that in a comparison between vinbarbital sodium and calcium ethyl 2-butyl N-methyl barbituric acid (hereafter called 8011), the LD_{50} in each case was higher in the males whereas no such difference was found in the ED_{50} in the two sexes. Also, the safety margin was higher with 8011, a fact which might mean either that 8011 was actually safer or this difference might have been due to the difference in age levels in the two determinations, in that with 8011 the rats were 3 to 6, whereas with vinbarbital sodium 6 to $8^1/_2$ months old.

EXPERIMENTAL

Our albino rats were originally bred from Wistar stock. The diet consisted of Purina Dog Chow and water. The rat quarters were kept at approximately 25° C. During the barbiturate tests, each rat was kept alone at 27–29° C. With vinbarbital sodium the rats were studied at four age levels, namely, 6 to $8^1/_2$, 3 to 5, 2 and 1 months; owing to the difficulty of securing a sufficient number of 2-month-old rats born within a few days, it was necessary to combine the rats of 2 week-end experiments and to supplement these experiments by adding the 112 and 141 mg./Kg. dosages on the following week end. Of the 6 to $8^1/_2$-month-old rats, over 90% were 7 to $8^1/_2$, and 60% were 8 to $8^1/_2$ months old; of the 3 to 5 month-old rats, nearly 60% were $3^1/_2$ to 4 months old; the 2-month-old rats were 53 to 63 days old, and nearly all 55 to 63 days old; the 1-month-old rats were 30 to 33 days old, with nearly 90% being 31 to 33 days old. With 8011, 48% were 5 to 6 and almost 80% were 4 to 6 months old. The older groups were deprived of food overnight; in case of

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the 2-month- and 1-month-old rats, most of the food was removed from the cages on the afternoon preceding the barbiturate tests, and the mothers were separated from the 1-month-old rats at the same time. The righting reflex was considered to be lost when a rat which had been picked up by the loose skin of the neck and tossed first upon its right and then upon its left side was unable to regain its front feet; this reflex was considered as recovered when a rat had regained its feet spontaneously and was able to crawl. This is essentially the technique employed by Hendrix (9). To insure maximum uniformity, all determinations of loss of the righting reflex were made by the same individual. An animal was considered fully recovered when it retracted either hind foot promptly from an extended position. A 2% solution of vinbarbital sodium (Lot No. x139778 in the first 3 and No. x230128 in the last of the determinations) in distilled water was freshly prepared usually twice for each day of the experiments; injections were made intraperitoneally. The solution of 8011 was prepared and injected in the same manner as delvinal sodium.

females 6.0 hrs.; with 63.1 mg./Kg. the values were 4.0 and 6.6 hrs., respectively; such differences were noted throughout. It was observed that among the females recovering in the higher dosage levels an unusually high proportion were severely depressed.

DISCUSSION

The results of our experiments may help to explain why different investigators have reported varying margins of safety for one and the same compound; such variations can be due to actual variation in rat stocks, but also to varying age of the rats used and of varying proportions of the two sexes or to choice of sex. Thus, in case of vinbarbital sodium, Hendrix (9) using adult male rats only, gives a ratio of 5.2; Cope, quoted from personal communication to Hendrix in above reference, reports a ratio of 4.3 and Molitor, similarly quoted, one of 4.0. Any one of these readily falls within the limits of the values secured in our studies, in which the range was 2.5 to 5.9, depending upon the age and sex. The most striking fact seems to be that whereas the dose causing loss of the righting reflex remained nearly

TABLE I.—SUMMARY OF EXPERIMENTS UPON THE EFFECTS OF AGE AND SEX UPON SAFETY MARGIN[a]

	Righting Reflex Lost														
	—Vinbarbital Sodium—												"8011"		
Age in Months:	6–8½			3–5			2			1			3–6		
Dose Range: Mg./Kg.	22–36			25–32			22–36			22–40			20–35		
No. of Groups ( )	(5)			(5)			(7)			(8)			(5)		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
Total number of rats:	39	39	78	40	40	80	56	56	112	72	72	144	30	30	60
ED_{50}, Mg./Kg.	28	27	27	28	27	28	29	28	28	29	29	29	26	26	26
	Mortality														
Dose Range: Mg./Kg.	40–159			50–200			79–200			89–224			62–188		
No. of Groups ( )	(13)			(13)			(9)			(9)			(9)		
Total number of rats:	100	100	200	104	104	208	84	84	168	81	81	162	54	54	108
LD_{50}, Mg./Kg.	80	66	71	120	119	120	167	157	163	173	173	173	117	85	100
LD_{50}/ED_{50}	2.9	2.5	2.6	4.3	4.4	4.3	5.7	5.6	5.7	5.9	5.9	5.9	4.5	3.3	3.8

[a] The two barbiturates were administered intraperitoneally as a 2% solution in distilled water. With vinbarbital sodium the dose increases were 12.2% (log 0.05), except that after the first test the two doses, 26.6 and 29.8 mg./Kg., were added at half of the previous logarithmic interval (5.93%) in the most critical part of the effective righting reflex depressing range. With calcium ethyl 2-butyl N-methyl barbituric acid ("8011") the dose increases were 15%. The ED_{50} represents the dose abolishing the righting reflex in 50% of the rats. The number of groups in each case was the same in the two sexes.

The results of the experiments with vinbarbital sodium are presented in Table I. With regard to the effect of age, the data show that whereas the dosage which causes loss of the righting reflex in 50% of the rats (ED_{50}, calculated by the Behrens' method (10)) remains nearly constant at the four age levels, the LD_{50} declines as age advances, especially above the 2-month level. Consequently, the margin of safety also declines distinctly, here from 5.9 to 2.6 at the extremes of the age range studied. Concerning sex, there is an unmistakable higher male resistance to vinbarbital sodium in the 6- to 8½-month group and to 8011 in the 3- to 6-month group, the only one studied with this barbiturate (Table I). It is puzzling that there was no sex difference seen in the LD_{50} in 3- to 5-month-old rats with vinbarbital sodium, because a study of the recovery times in the two sexes definitely favored the males; thus in case of 50.1 mg./Kg., the average recovery time for the 8 males was 3.8 and for the 8 constant in the 4 age groups and the 2 sexes, the average fatal dose declined distinctly with advancing age, so that, whereas in the younger animals 200 mg./Kg. of vinbarbital sodium was occasionally survived, in the group of 6- to 8½-month-old rats, the highest dose survived was only 126 mg./Kg. Only rats that seemed perfectly normal in appearance were included in these groups. In studies of this type it would seem pertinent for all authors reporting upon the margin of safety to give the sex and age range of the rats used in their determinations.

SUMMARY

1. Using a 2% solution of vinbarbital sodium (sodium 5-ethyl 5-(1-methyl 1-butenyl) barbituric acid) and intraperitoneal administration in white rats, the ratios of the LD_{50}/ED_{50} (latter representing the dose

abolishing the righting reflex in 50% of the rats) were 2.6 with 6 to $8^1/_2$ month-old rats, 4.3 with those 3 to 5 months old, 5.7 with 2-month and 5.9 with 1-month-old rats.

2. In case of hypnotic 8011 (calcium 5-ethyl 5-(2-butyl) N-methyl barbituric acid) the safety ratio was 4.5 with 3 to 6-month-old rats.

3. Whereas the ED_{50} with vinbarbital sodium remained between 27 and 29 mg./Kg., the LD_{50} declined from 173 in 1-month to 71 in 6- to $8^1/_2$-month-old rats.

4. In the oldest group with vinbarbital sodium the dose depressing the righting reflex was about equal in the 2 sexes, but the average fatal dose for the females was only 66 mg./Kg., as against 80 in the males, hence the safety margin was somewhat greater with the males. The same holds for 8011 with 3- to 6-month-old rats, in which the depressing dose was 26 mg./Kg. in either sex, but the LD_{50} was only 85 in the females as compared with 117 in the males, again giving a greater margin of safety for males.

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A Histological Study of *Chimaphila umbellata**

By Arthur E. Schwarting† and L. David Hiner††

The purpose of this investigation is the development of the histological description of the leaf of *Chimaphila umbellata* (Linné) Barton (Fam. *Ericaceae*) for the National Formulary monograph and a comparative description of the leaf of *Chimaphila maculata* (Linné) Pursh. (Fam. *Ericaceae*) as a means of distinguishing the two species. The leaf of *C. maculata* has frequently been substituted for the official National Formulary species and has been found as an adulterant in commercial samples (1). The entire herb of both species of Chimaphila has been used in medical practice both in America and Europe.

Chimaphila unbellata (*L.*) *Barton.*—This species has been known by many common English names, of which the most important are Pipsissewa, Bitter wintergreen, Pine tulip, Ground-holly, Bitter-sweet, Noble pine, Love-in-winter and Kings-cure. Chimaphila is the name generally used by taxonomists for the genus formerly included in the genus *Pyrola*, although this name still persists in the literature. The generic name is derived from two Greek words meaning winter loving, the species name describing the inflorescence. The species is a low perennial herb found in dry woods throughout the United States and Canada and also in Europe and Asia and remains green throughout the year.

All parts of the plant except the roots are endowed with the same active principles, the leaves with not more than 5% of stems being official in the National Formulary VII.

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Presented to the Scientific Section, A. PH. A., Denver meeting, 1942.

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Chimaphila umbellata (L.) Barton shows the characteristics of the genus *Chimaphila* of the *Ericaceae* family as follows: branches stout and leafy, extensively trailing, creeping or horizontally subterranean. Leaves spatulate or cuneate-oblanceolate, obtuse or acutish at the apex, sharply serrate, bright green and shining, not mottled, 2.5 to 7 cm. long, 8 to 20 mm. wide above the middle; flowers several, umbellate or subcorymbose, white or pinkish, commonly smaller than those of *C. maculata*, usually marked by a deep pink ring; filaments ciliate; capsule 6.4 to 8.5 mm. in diameter (2).

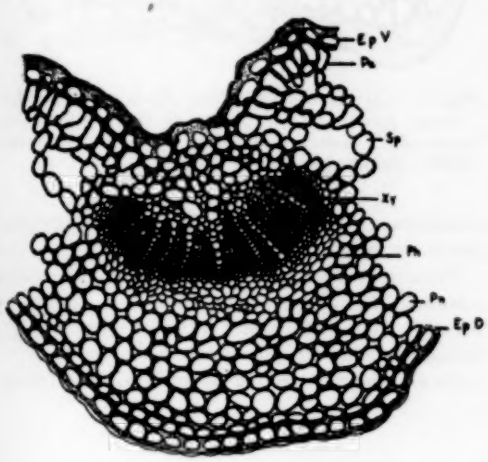


Fig. 1.—*Chimaphila umbellata* leaf, XS basal midvein.

Upper epidermis showing cutin layer (*EpV*); palisade (*Pa*); spongy parenchyma (*Sp*); fibrovascular bundle with trachea and wood fibers separated by medullary rays (*Xy*) with phloem (*Ph*) below and collenchyma above; colorless tissue (*Pn*); lower epidermis (*EpD*).

Chimaphila maculata (*L.*) *Pursh.*—This species has been referred to in English by many common names of which the most characteristic are Spotted pipsissewa, Rheumatism root, Dragon's-tongue, Wild arsenic and Ratsbane.

The plant has a geographical distribution similar to that of *C. umbellata* but the two species are seldom found growing together. The herb has been recognized by the North American Indians and the Eclectics as possessing medicinal properties similar to those of the species *C. umbellata*.

Chimaphila maculata (L.) Pursh. shows the characteristics of the genus *Chimaphila* of the *Ericaceae* family as follows: Stems extensively trailing, creeping or horizontally subterranean, sending up both sterile and flowering branches. Leaves lanceolate, ovate-lanceolate, or the lower much shorter and ovate, acute or acuminate at the apex, rounded or narrowed at the base, sharply serrate with rather distant teeth, dark green and mottled with white along the veins, 2.5 to 7.5 cm. long, 8 to 20 mm. wide below the middle; flowers few, corymbose or umbellate, white or pinkish; peduncle and pedicels puberulent; filaments villous at the middle; capsules erect, depressed-globose, about 8.5 mm. in diameter (3).

HISTORY

Chimaphila umbellata has long been used in this country and in Europe and is considered more valuable and used more frequently as a drug than *C. maculata*. The North American Indians recognized its properties as an antirheumatic and applied it externally in scrofula. A great deal has been written by the Eclectics who regard it as a neglected remedy that may well replace other agents of questionable safety.

"King's American Dispensatory" (4) states that *C. umbellata* is used in urinary disorders as a substitute for Uva Ursi and Buchu, to which it is preferable on account of being less obnoxious to the stomach.

The "Eclectic Materia Medica" (5) states that Chimaphila is both an antiseptic, diuretic and positive alterative. It does not dearrange but seems to favor digestion and has a good influence upon the processes of nutrition.

Chimaphila umbellata was first recognized by the United States Pharmacopœia in 1830 and retained until the Ninth Revision when it became official in the National Formulary IV (1916). It is an ingredient in the Fluidextract and Syrup Stillingia Compound.

EXPERIMENTAL

Discussion of the N. F. Monograph Chimaphila.—No paragraph on the structure of the leaf or stem of *C. umbellata* is included in the National Formulary monograph. To determine these structures microscopical studies were carried out upon specimens of *C. umbellata* obtained from the Pharmacognosy

Department of the Ohio State University, a commercial sample of the entire herb and a commercial sample of the drug labeled N. F. VI. Fresh specimens of the leaves and stems were collected in Hocking County, Ohio, of both species. Commercial samples of *C. maculata* were also obtained for the comparative study.

The samples of drug collected in Ohio were fixed in formalin-acetic acid-alcohol fixative and were embedded in paraffin. Transverse sections 10 μ in thickness were cut with a microtome throughout the length of the leaf and thus enabled an accurate study of the leaf structures. A differential stain of safranin and fast green was used to stain the tissues. Hand sectioning of the laboratory and commercial samples was made to confirm the structure of the microtomed sections and to study the cell elements which are masked by the staining process.

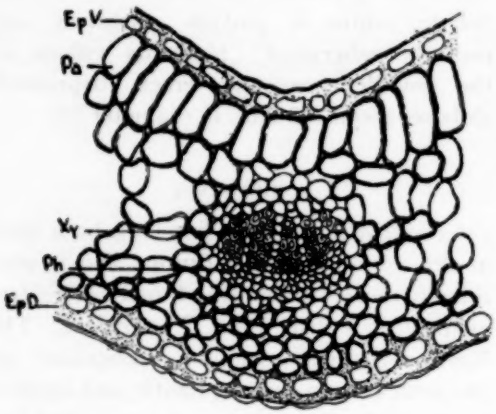


Fig. 2.—*Chimaphila umbellata*, XS apical midvein.

Upper epidermis (*EpV*); palisade (*Pa*); fibrovascular bundle with trachea and wood fibers (*Xy*); phloem (*Ph*); lower epidermis (*EpD*).

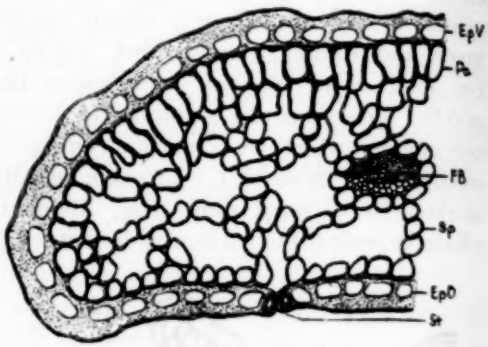


Fig. 3.—*Chimaphila umbellata*, XS lateral leaf edge.

Upper epidermis (*EpV*); palisade (*Pa*); fibrovascular bundle (*FB*); spongy parenchyma (*Sp*); lower epidermis (*EpD*); stomata (*St*).

Transverse Section of the Leaf of Chimaphila umbellata (See Figs. 1, 2, 3 and 4).—1. Upper epidermis: Cells with thickly cutinized outer walls and sinuous nodular, irregularly thickened, porous lateral walls and papillose above the midrib; no stomata.

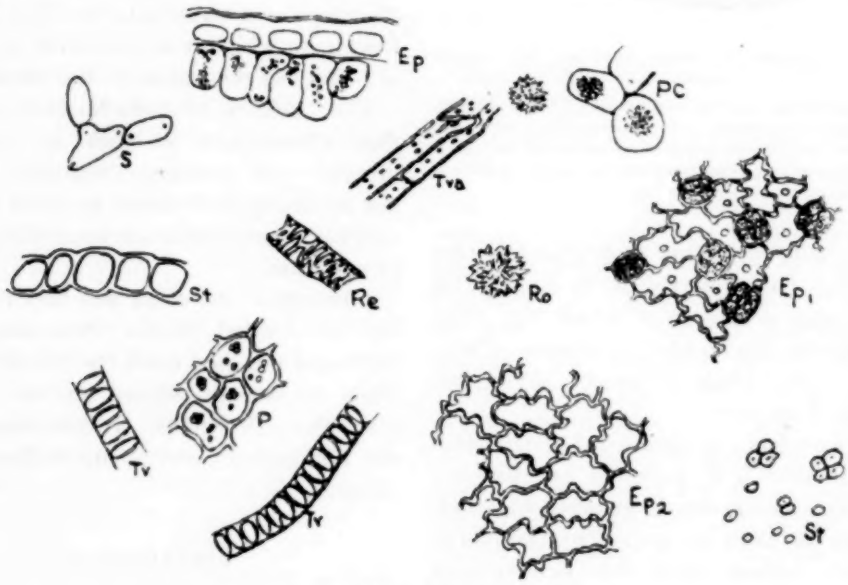


Fig. 4.—Powdered *Chimaphila umbellata*.

Upper epidermis with palisade parenchyma containing chloroplasts (*Ep*); spongy parenchyma (*S*); collenchymatous cells of stem (*St*); trachea (*Tr*); parenchyma with starch (*P*); reticulate ducts (*Re*); parenchyma with rosette aggregates (*PC*); tracheids (*tra*); rosette aggregate (*Ro*); starch (*St*); lower epidermis (Ep_1); upper epidermis (Ep_2).

2. Palisade parenchyma: A single row of compact columnar cells and a second loosely arranged layer of more nearly isodiametric cells rich in chloroplasts and occasionally containing a reddish brown to yellowish orange amorphous substance. The palisade layer is continuous across the leaf except above the midrib at the base of the leaf where it is replaced by a ridge of collenchyma.

3. Spongy parenchyma: Irregularly shaped cells with intercellular air spaces containing numerous rosettes of calcium oxalate; numerous fibrovascular bundles of the collateral type and arch shaped, with the xylem composed of trachea, tracheids and numerous highly lignified wood fibers

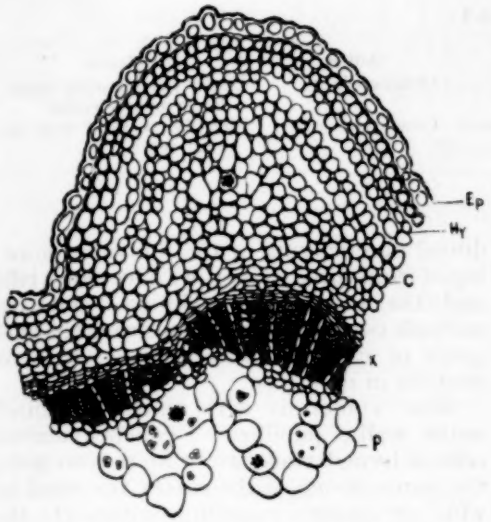


Fig. 5.—*Chimaphila umbellata* stem, transverse section.

Epidermis (*Ep*); hypodermis of collenchymatous cells (*Hy*); cortex (*C*); xylem fibers and trachea traversed by medullary rays (*X*); parenchyma (*P*).

separated by medullary rays one cell wide, each bundle surrounded by a layer of border parenchyma; the dorsal parenchyma of the midvein consisting of from 3 to 6 rows of colorless cells.

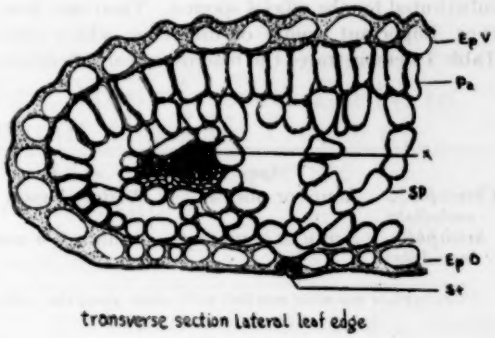
4. Lower epidermis: Cells with thickly cutinized outer walls and sinuous, irregularly thickened, porous lateral walls and papillose below the midrib; stomata numerous, broadly elliptical, up to 40 μ long, and nearly every guard cell containing a reddish brown to yellowish orange amorphous substance and each stomata bordered by 4 to 6 subsidiary cells.

Powdered Chimaphila umbellata (See Fig. 4).—The powdered elements exhibit the characteristics of both the leaf and stem. Since each guard cell of the stomata in the lower epidermis is bordered by from 4 to 6 subsidiary cells and nearly every guard cell contains an amorphous mass, it is suggested that these changes be noted in the official monograph.

Unground Chimaphila umbellata.—No mention is made of the type of venation occurring in the leaf other than that the veins are prominent. Although the cutin layer obscures the venation to some extent, it is distinctly finely reticulate on the upper surface and this reticulation is also seen on the lower epidermis with the larger veins more prominent.

Transverse Section of Stem of Chimaphila umbellata (See Fig. 5).—

1. Epidermis: Cells with thickly cutinized outer walls and papillose.



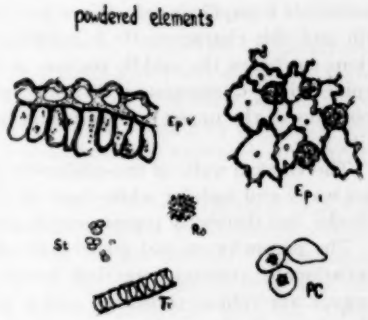


Fig. 6.—*Chimaphila maculata*.

Transverse section of leaf: Upper epidermis (*EpV*); palisade parenchyma (*Pa*); fibrovascular bundle (*X*); spongy parenchyma (*SP*); lower epidermis (*EpD*); stomata (*St*).

Powdered elements: Upper epidermis with adhering palisade cells and showing the papillose epidermis (*EpV*); starch (*St*); lower epidermis showing porous and beaded walls (*Ep*$_1$); rosette (*Ro*); parenchyma with rosette aggregates of calcium oxalate (*PC*); trachea (*Tr*).

2. Hypodermis: Thick-walled collenchymatous cells two or three rows wide.

3. Cortex: Rows of parenchyma cells traversed by very wide air spaces and containing numerous rosette aggregates of calcium oxalate.

4. Phloem: Polygonal cells, thin walled and some very large.

5. Cambium: A well-defined cambium does not exist although there is a distinct line of compact cells which separate the phloem and xylem.

6. Xylem: A complete cylinder of highly lignified woody tissue and radially arranged rays one cell wide. The scattered vessels are small isodiametric cells as compared to the polygonal and quadrangular wood fibers.

7. Pith: Large isodiametric parenchyma cells containing numerous simple and compound starch grains and rosette aggregates of calcium oxalate.

Comparative Description of Chimaphila maculata.—The leaf of *C. maculata* is in many respects similar to *C. umbellata*, and for this reason it is frequently substituted for the official species. There are, however, important points of difference which exist. Table I demonstrates the macroscopical differences.

TABLE I

	Apex	Base	Outline	Margin
Chimaphila umbellata	Acute or obtuse	Wedge-shaped	Oblanceolate	Upper part sharply serrate; lower nearly entire
himaphila maculata[a]	Acute or acuminate	Rounded or narrowed	Ovate-lanceolate	Sharply serrate with distant teeth

[a] *Chimaphila maculata* mottled with white along the veins.

Histological Comparison (See Fig. 6).—The outstanding histological differences between the leaf of *C. maculata* and *C. umbellata* is exhibited in the epidermal layers. The cutinized epidermis of *C. umbellata* is papillose only above and below the midrib and this characteristic is exhibited only in sections cut below the middle portion of the leaf. The epidermis of *C. maculata* is distinctly papillose on all portions of the upper epidermis and below the midrib.

The vertical walls of the epidermis of *C. umbellata* are wavy and nodular while those of *C. maculata* are similar but distinctly porous and beaded.

The parenchyma and guard cells of both species occasionally contain a reddish brown to yellowish orange amorphous substance and it cannot be said that it predominates in one species over the other.

CONCLUSIONS

The changes and additions to the National Formulary monograph Chimaphila are suggested to read as follows:

Unground Chimaphila—Oblanceolate, from 2.5 to 7 cm. long and from 8 to 20 mm. wide, *apex acute or somewhat obtuse, base wedge shaped*, the upper portion coarsely and sharply serrate, the lower nearly entire; coriaceous, smooth and uniformly light olive-brown to dark yellow-green, *finely reticulate on the upper surface*, paler beneath, the veins prominent.

Histology—*Leaf*, upper epidermis with wavy and nodular vertical walls, cuticle much thickened and somewhat papillose above the midrib (*C. maculata* distinctly papillose) and no stomata; vertical walls of lower epidermis wavy and nodular, cuticle thickened and numerous broadly elliptical stomata up to 40 microns long and nearly every guard cell containing an amorphous substance; a palisade layer consisting of a single row of columnar cells and a second layer of more nearly isodiametric cells extending across the midrib but replaced by a characteristic ridge of collenchyma at the base of the leaf. The vascular bundles in the midrib and petiole arch shaped, composed of a strongly developed xylem region with medullary rays one cell wide. The dorsal parenchyma of the midvein consisting of from three to six rows of colorless cells and the spongy parenchyma below the palisade containing numerous rosette aggregates of calcium oxalate from 25 to 65 microns in diameter.

Stem, epidermis with thickly cutinized outer walls, papillose; collenchymatous cells of hypodermis two or more rows wide; the parenchyma of the cortex traversed by wide air spaces; cambium indistinct; the xylem a complete cylinder of radially arranged wood fibers and trachea with medullary rays one cell wide; the pith composed of large isodiametric parenchyma cells containing numerous starch granules and rosette aggregates of calcium oxalate.

Powdered Chimaphila—Color moderate yellowish brown to light olive; odor slight; taste astringent and bitter; epidermal cells with thickly cutinized outer walls and sinuous, irregularly thickened, porous lateral and inner walls, the lower epidermis with broadly elliptical stomata up to 40 microns long and *bordered by four to six subsidiary cells*; parenchyma *and guard cells of stomata* occasionally containing a reddish brown to yellowish orange amorphous substance; tracheae spiral and annular, together with elongated, thick-walled, lignified cells showing numerous minute reticulations; calcium oxalate aggregates up to 65 microns in diameter, mostly about 35 microns; starch grains few, rounded, simple, up to 16 microns in diameter, sometimes with a central cleft, or 2- to 4-compound; few lignified fibers and epidermal fragments from the stems and the roots.

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(2) Britton, N. L., and Brown, A., "An Illustrated Flora of the Northern United States, Canada and the British Possessions," Charles Scribner's Sons, New York, N. Y., Vol. II, 1936, p. 672.

(3) *Ibid.*, p. 2.

(4) Felter, H. W., "King's American Dispensatory," The Ohio Valley Company, Cincinnati, Ohio, 1856, p. 334.

(5) Felter, H. W., "Eclectic Materia Medica, Pharmacology and Therapeutics," J. K. Scudder, Cincinnati, Ohio, 1922, p. 228.

The Effect on Estrus of Drugs Administered Daily in Therapeutic Doses Throughout the Life Cycle of Albino Rats and the Estrus Cycle Sequence with Reference to Age*

By Lloyd L. Boughton and O. O. Stoland

Earlier papers have presented the effects of life-cycle administration of commonly used drugs on general welfare, behavior and death rate (1), and on activity, maze learning and relearning (2).

All drugs investigated were fed orally to thoroughly tamed rats of the Wistar strain which were raised in our own laboratory. Drugs were administered in doses approximating, on a weight basis, the average daily therapeutic dose for adult humans.

EXPERIMENTAL

The estrus cycles of 88 rats in 11 test and control groups were determined by the method suggested by Long and Evans (3). Vaginal smears were prepared by introducing the tip of a small medicine dropper containing a drop of normal saline just inside the lip of the vagina and carefully compressing and releasing the bulb. The material thus obtained was transferred to a slide and examined under the low power of a microscope. The daily examination of smears was started regularly at 9 a. m.

Long and Evans (3) state that the succession of cell types occurring in the vaginal smear at various stages during the estrus cycle is invariable ,and that the stages may be appropriately named from the cell content of the smear as follows:

1. The stage of the sudden appearance of masses of uniform-size, nucleated epithelial cells dehisced from the surface.
2. The stage of few large cornified cells.
3. The stage of extremely abundant cornified cells.
4. The stage of many leucocytes admixed with cornified cells.
5. Stage of leucocytes with scanty epithelial cells.

According to Long and Evans, rats are definitely in heat during all of stage 2 and may accept the male during the late part of stage 1 or early part of stage 3. The complete cycle, according to these authors, extends over four days and is extremely regular in sequence.

Tables I, II, III and IV present the data obtained from test and control groups at different periods during the life cycle of the animals. Each table gives the age in weeks, the time of year and the length of each observation period in days. Drug feedings were started in all test groups at the age of 10 weeks. The number of weeks of drug administration may therefore be determined by subtracting 10 from the stated age at any observation period. Litter mates were used as controls and all rats represented in each table were from the same litters.

Although there is considerable discussion of the estrus cycle of albino rats in the literature, the authors have failed to find any papers that present actual data with reference to varying lengths of cycles, of intervals between cycles, etc. It is for this reason that data are presented rather completely for individual animals in the tables included in this paper. Other papers on the subject have failed even to give the ages of animals from which smears were obtained, and, to our knowledge, there have been no statements concerning cycle sequence with reference to age.

There is very little in the literature to indicate the effect of drugs on the estrus cycle. Branch and Moss (4) have used this rhythmical sequence as a criterion for determining the effect of nicotine ad-

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ministration on female white rats. These authors report that the number of 4-day cycles occurring during a given experimental period is reduced by nicotine medication. Their evidence is, however, far from conclusive.

the fact that one of the aspirin rats experienced only 6 cycles during the 202 days of observation, 4 of which were 4-day cycles. There are no significant differences if this animal is not considered. Comparisons for the first 4 periods show no significant

TABLE I.—ESTRUS CYCLES OF AMINOPYRINE ANTIPYRINE GROUP

No.	Four-Day Cycles of Total Cycles for Each of 4 Periods 1st, 10 to 16 Wks., Jan.–Feb., 42 Days	2nd, 27 to 31 Wks., May–June, 30 Days	3rd, 61 to 65 Wks., Jan.–Feb., 31 Days	4th, 72 to 76 Wks., Mar.–Apr., 28 Days
	Aminopyrine Animals, Nos. 1 to 9			
1	9 of 10	6 of 8	5 of 6	2 of 4
2	9 of 11	Dead	...	...
3	8 of 9	4 of 7	1 of 3	0 of 2
4	7 of 9	6 of 8	3 of 5	0 of 1
5	8 of 9	0 of 2	0 of 3	0 of 0
6	8 of 10	6 of 7	4 of 5	0 of 0
7	7 of 9	3 of 4	0 of 0	0 of 1
8	8 of 9	0 of 1	0 of 0	0 of 2
9	7 of 10	6 of 6	1 of 2	0 of 0
Av.	8.0–9.6	4.0–5.4	1.7–3.0	0.25–1.2
	Control Animals, Nos. 1 to 9			
1	10 of 10	4 of 7	Dead	...
2	7 of 8	5 of 9	1 of 3	2 of 3
3	8 of 9	6 of 9	3 of 5	0 of 3
4	6 of 9	1 of 3	4 of 6	0 of 0
5	9 of 10	4 of 5	Dead	...
6	7 of 9	6 of 7	0 of 2	0 of 0
7	8 of 9	0 of 0	1 of 3	0 of 1
8	6 of 7	6 of 7	0 of 0	0 of 0
9	9 of 10	5 of 7	Dead	...
Av.	8.0–9.0	4.1–6.0	1.5–3.2	0.33–1.2
	Antipyrine Animals, Nos. 1 to 9			
1	8 of 9	4 of 6	Dead	...
2	8 of 9	0 of 2	0 of 3	1 of 4
3	10 of 10	6 of 6	5 of 7	0 of 1
4	10 of 10	7 of 8	0 of 0	0 of 0
5	8 of 9	1 of 4	0 of 1	1 of 2
6	10 of 10	4 of 6	1 of 3	Dead
7	7 of 9	4 of 7	Dead	...
8	8 of 10	5 of 6	2 of 4	0 of 1
9	6 of 8	3 of 5	0 of 4	0 of 2
Av.	8.4–9.3	3.8–5.6	1.2–3.2	0.33–1.7

Animals Showing No Cycles during Periods

	First	Second	Third	Fourth
Aminopyrine	0 of 9	0 of 8	2 of 8	3 of 8
Controls	0 of 9	1 of 9	1 of 6	3 of 6
Antipyrine	0 of 9	0 of 9	1 of 7	1 of 6

Average total cycles for rats present through all 4 periods (131 days).
Aminopyrine 19.5 (8 rats), *Controls* 18.7 (6 rats), *Antipyrine* 19.7 (6 rats).
Average 4-day cycles for rats present through all periods.
Aminopyrine 13.5 (71%), *Controls* 13.0 (69%), *Antipyrine* 13.5 (70%).

None of the drugs studied during this investigation have had a significant effect on the estrus cycle, with the possible exception of aspirin. As observed in Table II, all 3 aspirin-fed animals surviving the fifth period failed to show any evidence of heat during this period. During this same period, only 1 of 4 controls and 2 of 5 sodium phenobarbital animals failed to do so. The groups compare favorably in this respect, however, during the fourtn period.

The average total cycles and average 4-day cycles appear to be significantly lower for the aspirin animals than for the controls, but attention is called to variations in total or 4-day cycles.

One animal in the sodium alurate group (Table III) experienced only two 4-day cycles during 128 days of observation.

Blood was found almost consistently in the smears of 2 of the aminopyrine animals (Nos. 6 and 9, Table I) and less consistently in the smears of one antipyrine animal (No. 4, Table I).

Normal Cycle Sequence versus Age.—Of 76 animals examined below the age of 25 weeks, none failed to exhibit estrus cycles. Eighty-four per cent (590 of 698 cycles) were 4-day cycles.

TABLE II. ESTRUS CYCLES OF ASPIRIN SODIUM PHENOBARBITAL GROUP

No.	1st, 17 to 24 Wks., Nov.–Dec., 50 Days	2nd, 26 to 33 Wks., Jan.–Feb., 42 Days	3rd, 43 to 47 Wks., May–June, 30 Days	4th, 62 to 68 Wks., Sept.–Oct., 40 Days	5th, 86 to 92 Wks., Mar.–Apr., 40 Days
	Four-Day Cycles of Total Cycles for Each of 5 Periods				
	Aspirin Animals, Nos. 1 to 8				
1	9 of 10	7 of 8	6 of 8	2 of 5	0 of 0
2	10 of 10	6 of 9	5 of 7	0 of 2	Dead
3	9 of 10	9 of 9	0 of 0	0 of 3	Dead
4	12 of 12	3 of 5	3 of 6	1 of 3	Dead
5	12 of 12	4 of 7	4 of 6	Dead	...
6	10 of 11	8 of 11	3 of 5	Dead	...
7	4 of 5	0 of 1	0 of 0	0 of 0	0 of 0
8	7 of 9	6 of 9	2 of 3	4 of 6	0 of 0
Av.	9.1–9.9	5.5–7.4	3.0–4.4	1.2–3.2	0–0
	Control Animals, Nos. 1 to 7				
1	7 of 10	2 of 5	4 of 7	2 of 4	0 of 2
2	9 of 11	8 of 9	3 of 5	1 of 3	Dead
3	7 of 8	5 of 7	0 of 0	Dead	...
4	10 of 11	7 of 8	3 of 5	4 of 6	1 of 5
5	7 of 9	6 of 9	0 of 0	0 of 0	0 of 0
6	11 of 11	9 of 9	4 of 6	0 of 3	0 of 3
7	11 of 12	0 of 1	0 of 0	Dead	...
Av.	8.9–10.3	5.3–7.0	2.0–3.3	1.4–3.2	0.25–2.5
	Sodium Phenobarbital Animals, Nos. 1 to 7				
1	11 of 11	4 of 5	5 of 7	0 of 2	0 of 0
2	7 of 10	8 of 9	4 of 7	0 of 1	5 of 8
3	12 of 12	7 of 9	1 of 4	0 of 0	Dead
4	11 of 11	4 of 8	3 of 5	2 of 4	0 of 2
5	6 of 8	3 of 6	0 of 0	3 of 5	0 of 0
6	11 of 11	7 of 9	0 of 1	3 of 4	Dead
7	11 of 12	6 of 9	5 of 8	0 of 2	0 of 2
Av.	9.9–10.3	5.6–8.0	2.6–4.6	1.1–2.6	1.0–2.4

Animals Showing No Cycles during Periods

	First	Second	Third	Fourth	Fifth
Aspirin	0 of 8	0 of 8	2 of 8	1 of 6	3 of 3
Controls	0 of 7	0 of 7	3 of 7	1 of 5	1 of 4
Na phenobarbital	0 of 7	0 of 7	1 of 7	1 of 7	2 of 5

Average total cycles for animals present through all 5 periods (202 days).
Aspirin 21.3 (3 rats), *Controls* 28.2 (4 rats), *Na Phenobarbital* 28.4 (5 rats).
Average 4-day cycles for rats present through all periods.
Aspirin 15.7 (73%), *Controls* 19.2 (68%), *Na Phenobarbital* 19.6 (70%).

Seven per cent of the animals examined between the ages of 25 and 50 weeks failed to show evidence of heat. Seventy per cent of the cycles (526 of 743) obtained between these ages were 4-day cycles.

Between 50 and 75 weeks of age, 21% of animals examined failed to show heat. Only 44% (81 of 186) of the cycles obtained during this age interval were 4-day cycles.

Twenty-five of 57 animals examined beyond the age of 75 weeks (44%) failed to exhibit estrus. Only 29% of the cycles occurring after 75 weeks of age were 4-day cycles.

In our experience, albino rats of the Wistar strain exhibit a sequence of 4-day cycles with marked regularity up to about 50 weeks of age. Beyond 50 weeks the smears become increasingly difficult to

TABLE III.—ESTRUS CYCLES OF ALLONAL SODIUM ALURATE GROUP

No.	1st, 19 to 25 Wks., Jan.–Feb., 40 Days	2nd, 35 to 39 Wks., May–June, 30 Days	3rd, 70 to 74 Wks., Jan.–Feb., 31 Days	4th, 80 to 84 Wks., Mar.–April, 27 Days
	Four-Day Cycles of Total Cycles for Each of 4 Periods			
	Allonal Animals, Nos. 1 to 9			
1	4 of 8	4 of 4	Dead	...
2	7 of 9	Dead	...	...
3	7 of 8	5 of 7	2 of 3	0 of 2
4	6 of 9	5 of 8	3 of 5	0 of 2
5	9 of 10	6 of 6	0 of 1	0 of 0
6	5 of 9	5 of 7	0 of 1	1 of 2
7	9 of 10	4 of 7	0 of 0	0 of 0
8	6 of 8	2 of 5	Dead	...
9	8 of 9	5 of 7	1 of 3	0 of 0
Av.	6.8–9.0	4.5–6.4	1.0–2.2	0.17–1.0

TABLE III.—ESTRUS CYCLES OF ALLONAL SODIUM ALURATE GROUP. (*Continued.*)

	Four-Day Cycles of Total Cycles for Each of 4 Periods: 1st, 19 to 25 Wks., Jan.–Feb., 40 Days	2nd, 35 to 39 Wks., May–June, 30 Days	3rd, 70 to 74 Wks., Jan.–Feb., 31 Days	4th, 80 to 84 Wks., Mar.–April, 27 Days
Control Animals, Nos. 1 to 9				
1	8 of 9	5 of 6	0 of 1	0 of 1
2	7 of 8	5 of 6	0 of 0	0 of 0
3	6 of 8	4 of 7	Dead	...
4	7 of 10	3 of 5	Dead	...
5	9 of 10	4 of 8	2 of 3	0 of 1
6	4 of 8	0 of 3	Dead	...
7	8 of 8	5 of 6	0 of 1	0 of 0
8	2 of 6	5 of 7	1 of 4	2 of 3
9	7 of 9	0 of 2	Dead	...
Av.	6.4–8.4	3.4–5.5	0.6–1.6	0.4–1.0
Sodium Alurate Animals, Nos. 1 to 9				
1	7 of 9	4 of 5	0 of 0	0 of 0
2	6 of 8	5 of 7	0 of 0	0 of 2
3	2 of 4	0 of 3	0 of 2	0 of 0
4	5 of 8	Dead	...	...
5	9 of 10	6 of 6	2 of 4	0 of 1
6	8 of 11	5 of 7	0 of 2	2 of 4
7	4 of 7	0 of 0	Dead	...
8	6 of 9	2 of 5	6 of 7	0 of 1
9	7 of 9	Dead	...	...
Av.	6.0–8.3	3.3–4.7	1.3–2.5	0.33–1.3

Animals Showing No Cycles during Periods

	First	Second	Third	Fourth
Allonal	0 of 9	0 of 8	1 of 6	3 of 6
Controls	0 of 9	0 of 9	1 of 5	2 of 5
Na Alurate	0 of 9	1 of 7	2 of 6	2 of 6

Average total cycles for rats present through all 4 periods (128 days).
Allonal 18.7 (6 rats), *Controls* 17.6 (5 rats), *Na Alurate* 17.5 (6 rats).
Average 4-day cycles for rats present through all 4 periods.
Allonal 13.5 (70%), *Controls* 12.6 (72%), *Na Alurate* 11.7 (67%).

TABLE IV.—ESTRUS CYCLES OF CAFFEINE GROUP

No.	Four-Day Cycles of Total Cycles for Each of 5 Periods: 1st, 34 to 43 Wks., Oct.–Dec., 61 Days	2nd, 46 to 52 Wks., Jan.–Feb., 42 Days	3rd, 62 to 66 Wks., May–June, 30 Days	4th, 81 to 87 Wks., Sept.–Oct., 40 Days	5th, 108 to 112 Wks., May–Apr., 20 Days
Caffeine Animals, Nos. 1 to 7					
1	9 of 13	5 of 7	0 of 0	1 of 2	0 of 0
2	9 of 13	7 of 10	0 of 0	0 of 0	1 of 2
3	6 of 10	3 of 5	0 of 1	Dead	...
4	7 of 11	4 of 7	3 of 5	4 of 7	0 of 0
5	11 of 14	6 of 9	0 of 1	0 of 0	0 of 0
6	10 of 13	7 of 10	4 of 6	3 of 4	0 of 1
7	8 of 10	4 of 6	4 of 6	Dead	...
Av.	8.6–12.0	5.1–7.7	1.6–2.7	1.6–2.6	0.2–0.6
Control Animals, Nos. 1 to 5					
1	9 of 12	8 of 10	3 of 5	0 of 2	0 of 0
2	12 of 15	9 of 11	0 of 0	3 of 5	0 of 3
3	7 of 11	7 of 9	0 of 3	0 of 0	0 of 0
4	11 of 13	4 of 5	2 of 4	0 of 2	Dead
5	9 of 12	5 of 7	Dead	...	...
Av.	9.6–12.6	6.6–8.4	1.3–3.0	0.8–2.3	0–1.0

Animals Showing No Cycles during Periods

	First	Second	Third	Fourth	Fifth
Caffeine	0 of 7	0 of 7	2 of 7	2 of 5	3 of 5
Controls	0 of 5	0 of 5	1 of 4	1 of 4	2 of 3

Average total cycles for rats present through all 5 periods (192 days).
Caffeine 27 (5 rats), *Controls* 29 (3 rats).
Average 4-day cycles for rats present through all periods.
Caffeine 18 (70%), *Controls* 19 (67%).

read because of the fact that the transition from stage to stage becomes rather rapidly less marked. We frequently found animals exhibiting a mixture of all types of cells day after day for weeks at a time, with no outward signs of being in heat. The stage of abundant cornified cells disappeared completely in many cases, resulting in smears containing only nucleated epithelial cells and leucocytes.

The females in all groups were mated with litter mate males for 2 weeks at 70 weeks of age. Although 53 of 66 females were showing evidences of heat as determined by the smear method during this period, no young were obtained, due certainly to no lack of aggression on the part of the males. Copulation was observed in a few instances, but the females generally fought off the attentions of the males.

SUMMARY

1. Prolonged medication in albino rats with aminopyrine, antipyrine, aspirin, sodium phenobarbital, sodium alurate, allonal and caffeine, with daily doses approximating, on a weight basis, the average daily therapeutic dose for the adult human, has produced no significant variations in the estrus cycles of test animals as compared to litter mate controls.

2. The sequence of 4-day cycles is markedly regular up to about 50 weeks of age but becomes increasingly irregular beyond this age.

3. All animals examined below the age of 25 weeks exhibited estrus cycles.

4. Seven per cent of animals examined between ages of 25 and 50 weeks, 21% between 50 and 75 weeks and 44% beyond 75 weeks failed to show evidence of heat by smear method.

Eighty-four per cent of cycles occurring in animals under 25 weeks of age were 4-day cycles. The other age groups experienced, respectively, 70, 44 and 29% of 4-day cycles.

REFERENCES

(1) Boughton, L. L., and Stoland, O. O., *Univ. of Kans. Sci. Bull.*, 27 (1941), 27.

(2) Boughton, L. L., THIS JOURNAL, 31 (1942), 240.

(3) Long, J. A., and Evans, H. M., *Mem. Univ. Calif.*, 6 (1922), 1.

(4) Branch, H. E., and Moss, W. G., *Trans. Kans. Acad. Sci.*, 41 (1938), 317.

Books Received (*Continued*)

To April 1, 1943

HAROLD N. WRIGHT and MILDRED MONTAG, *Textbook of Materia Medica, Pharmacology and Therapeutics.* Second edition. W. B. Saunders, Co., West Washington Square, Philadelphia, 1942. 647 pp. $3.00.

HEBER W. YOUNGKEN, *Textbook of Pharmacognosy.* Fifth edition. The Blakiston Company, Philadelphia, 1943. 1038 pp. $7.50.

E. WERTHEIM, *Experiments in Organic Chemistry into Some Biochemistry Experiments.* The Blakiston Company, 1012 Walnut St., Philadelphia, 1942. 221 pp. $1.35.

D. WRIGHT WILSON, *Laboratory Manual of Physiological Chemistry.* Fourth edition. Williams and Wilkins, Mount Royal and Guilford Aves., Baltimore, Md., 1941. 298 pp. $2.50.

Book Reviews

Chemistry of Insecticides and Fungicides, by DONALD E. H. FREAR. D. Van Nostrand Company, Inc., 250 Fourth Avenue, New York, N. Y., 1942. viii + 300 pp., 31 figs., 15.5 x 23.5 cm. Price, $4.00.

This book, according to the author's statement, is the outgrowth of his lecture notes and literature references used in a course in the chemistry of insecticides and fungicides given at the graduate level in The Pennsylvania State College.

As a text and reference work it is concerned solely with the chemistry of the natural and synthetic agents used to control insects and plant diseases. It marks a commendable departure from the trend in this field which has been to provide a single publication covering the subjects of applied chemistry, entomology, spraying methods, etc.

The material is divided into 5 principal sections: Part I—Stomach Poisons; Part II—Contact Poisons; Part III—Fungicides; Part IV—Spray Supplements and Residue Removal; Part V—Analytical Methods. The chemical agents are classified according to their action(s) with respect to the headings of the first 4 sections. In those cases where a single compound is known to have more than one action it appears under the several different headings, but with the proper emphasis in each instance. The fifth section is composed of macro and micro analytical methods used with insecticides, fungicides, etc. The methods are reprinted from *Official and Tentative Methods of Analyses* of the A. O. A. C., Fifth edition, 1940.

The author covers the subject matter well, and moreover he does it with conciseness and clarity. The other admirable features of this book are its up-to-date bibliography at the end of each chapter, the author index and the use of figures to emphasize certain portions of the material. The printing is clear, the paper is of a good quality and the binding is attractive.

There are, however, several ambiguous statements, incomplete definitions and typographical errors which detract from this valuable publication. The modern spelling of the word "homologue" (p. 8) is to be preferred. While the amount of calcium arsenate used in this country (p. 12) varies widely from year to year, it is certainly not declining. The solubility of As_2O_5 in water (p. 21) is considerably higher than that indicated by the author. The name "Staudinger" (p. 65) is spelled without the letter *g*. This reviewer can find nothing to indicate that quassin and its isomers neoquassin and picrasmin (p. 88) are related to rotenone in chemical structure. Reference no. 46 on page 88 and again on page 89 is in error. The definition of soaps as "the alkali salts of fatty acids" (p. 126) is too restricted as indicated by the author's own reference to lead and calcium soaps on page 130. A good fungicide is lethal in low concentrations to pathogens, and not "to pathogens in low concentrations" as stated on page 153. The saponins are more correctly defined (p. 185) as glycosides and not as glucosides. The word "effervescence" (p. 247, line 19) is incorrectly spelled.

This book is highly recommended as a text for students and as a reference book for practicing pharmacists, plant pathologists, economic entomologists and others who are interested in the chemical agents used to eradicate insects and control plant diseases.—W. T. SUMERFORD

Text-Book of Pharmacognosy, by HEBER W. YOUNGKEN. The Blakiston Company, Philadelphia, Pa., 1943. xiv + 1038 pp. Price, $7.50.

The general plan of this work is just about the same as in former editions, but many pages have been completely revised and considerable new material has been added. Several new drug monographs and many illustrations have been introduced. Some of the old illustrations have been replaced by more complete ones. Due to the results of investigations in the field of pharmacognosy several changes in the methods of production of drugs have been made. Many crude drugs have found other uses in the last few years, and the author has brought the book up to date in this direction. The United States Pharmacopœia and the National Formulary have been revised since the last edition and, of course, the author has brought the book up to date in this respect. Due to revisions adopted by the International Botanical Congresses, some changes in the naming of species of drug plants have been necessary. Because of the scarcity of crude drugs formerly imported from Europe and other remote parts of the world, the interest in the cultivation of drug plants in this country has been greatly increased. To aid in this work the author has included suggestions on the cultivation, harvesting, drying and storage of such drugs. No doubt this edition will help many in the practical side of pharmacognosy.

In the chapter on drugs of animal origin, fifteen pages of new material have been added to take care of drugs of this origin which have either become official or have been recently introduced into medicine.

In this as in previous editions the author has given the chief pharmacological actions of the drugs. In order to assist the student in understanding these actions, the author has included a glossary of therapeutic terms. The official doses are also included—C. J. ZUFALL.